Fundamental Studies on the Current-Voltage Curve in Polarography. I.*

Derivation of the Diffusion-Controlled Polarographic Waves of Simple Metal Ions from the Standpoint of Chemical Kinetics.

By Nobuyuki TANAKA and Reita TAMAMUSHI.

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In polarography, the formula of the current-voltage curve has been derived and the conception of the half-wave potential has been introduced by J. Heyrovský and D. Ilkovič[1] in 1935. In this case, as the base of derivation, it was set down that the electrode potential is given by the Nernst's equation and the current is determined by the rate of diffusion of reducible or oxidizable substances. However, since the Nernst's equation, which is derived in thermodynamics, gives the static electrode potential, its application to polarography, in which the electrode potential seems to be dynamic, appears doubtful. Even if the application of the Nernst's equation to polarography is admitted as an approximation, the formula of the current-voltage curve obtained in that case is naturally confined to that of the "reversible wave". The "irreversible wave" obtained in most cases in polarography is impossible to be treated theoretically with the formula based on the Nernst's equation.

The new derivation of the formula of the current-voltage curve in polarography has been carried out by the present authors from viewpoint of chemical kinetics.[2] In other words the formula has been derived from the relation between the potential difference of electrode and solution and the current, independently of the Nernst's equation. In this treatment, the "irreversible wave" can be taken into consideration as well as the "reversible wave" and the Nernst's equation is proved to be fulfilled under particular conditions as the approximation.

The derivation of the formula has been carried out for the deposition of metals from the simple (or hydrated) metal ions, the oxidation-reduction of simple (or hydrated) metal ions, the reduction or the oxidation-reduction of organic compounds and the anodic current resulting from the dissolution of the dropping mercury electrode. Of these, in

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(1) J. Heyrovský and D. Ilkovič, *Collection Czechoslovak Chemical Communications*, **7** (1935), 198.

(2) Glasstone, Laidler and Eyring, "The Theory of Rate Processes", McGraw-Hill (1941); G. E. Kimball, *J. Chem Phys.*, **8** (1940), 199.

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this paper, the former two under the conditions of the diffusion-control have been dealt with, in which, since the oxidation-reduction of simple (or hydrated) metal ions gives the more general formula, the formula for it is derived first and that for the deposition of metal is represented as one in particular conditions. The reduction or the oxidation-reduction of organic compounds is to be reported in the succeeding papers, as well as the electrode reaction of the complex metal ions and, further, the various electrode reactions under the conditions of the non-diffusion-control. As for the dissolution of the dropping mercury electrode the derivation of the formula has been furnished by Eyring[2] in the more general case of the dissolution of metals.

Oxidation-reduction of simple (or hydrated) metal ions. Consider the net electrode reaction represented by the equation (1), in which adsorption, decomposition, polymerisation and the like are not assumed to occur at the electrode surface.

$$\mathrm{M}^{m+} + n\ominus \rightleftarrows \mathrm{M}^{(m-n)+}, \qquad (1)$$

where M^{m+} and $\mathrm{M}^{(m-n)+}$ symbolize the oxidized and the reduced forms of simple (or hydrated) metal ions respectively. Consider a potential-energy barrier to exist between the initial state, the oxidized form M^{m+}, and the final state, the reduced form $\mathrm{M}^{(m-n)+}$. If the free energy difference of the initial and the activated states and that of the final and the activated states are represented by ΔF_1 and ΔF_2 respectively, the difference of ΔF_1 and ΔF_2 becomes the free energy difference of the initial and the final states, which is represented by ΔF_0.

Assume that the reaction in which the oxidized form is reduced at the electrode surface is the forward reaction and the reaction in which the reduced form is oxidized at the electrode surface is the reverse reaction, and that the potential difference between the electrode and the solution, V, acts completely between the initial and the final states of the electrode reaction. If α is the fraction of the potential V that is operative between the initial and the activated states, the potential αV will facilitate the forward reaction and $(1-\alpha)\,V$, retard the reverse reaction, and then the rates of the forward and the reverse reactions are represented by the equation (2)

$$\begin{aligned} &\text{forward reaction:} \quad s\cdot\frac{kT}{h}[A_O]\,e^{-\Delta F_1/RT}\,e^{-n\alpha VF/RT} = [A_O]\,\kappa_1 E_1 \\ &\text{reverse reaction:} \quad s\cdot\frac{kT}{h}[A_R]\,e^{-\Delta F_2/RT}\,e^{n(1-\alpha)VF/RT} = [A_R]\,\kappa_2 E_2, \end{aligned} \qquad (2)$$

where s is the constant which depends on the nature of the electrode, $[A_O]$ and $[A_R]$ represent the activities of the oxidized and reduced forms

at the electrode surface respectively, and κ_1, κ_2, E_1 and E_2 are the abbreviations of $s\frac{kT}{h}e^{-\Delta F_1/RT}$, $s\frac{kT}{h}e^{-\Delta F_2/RT}$, $e^{-n\alpha VF/RT}$ and $e^{n(1-\alpha)VF/RT}$ respectively. In the equation (2) it is also considered that in the reaction concerned the forward reaction is facilitated when the electrode potential becomes more negative.

Since the magnitude of current flowing is determined by the difference of the two rates, the current, assumed to be cathodic, is

$$I = n\,\varepsilon\,\{[A_O]\,\kappa_1 E_1 - [A_R]\,\kappa_2 E_2\}. \tag{3}$$

On the other hand, since, in polarography, the current flowing depends almost entirely on the diffusion because of the existence of large excess of indifferent electrolytes, it can be represented by the diffusion equations for the oxidized and the reduced forms, as mentioned below:

$$I = n\varepsilon K_O([C_O]^\circ - [C_O]) = n\varepsilon K_O/f_O([A_O]^\circ - [A_O]) = n\varepsilon K_O'([A_O]^\circ - [A_O]) \tag{4}$$

$$I = n\varepsilon K_R([C_R] - [C_R]^\circ) = n\varepsilon K_R/f_R([A_R] - [A_R]^\circ) = n\varepsilon K_R'([A_R] - [A_R]^\circ) \tag{5}$$

$[C_O]$, $[C_R]$: concentrations of the oxidized and reduced ions at the electrode surface.

$[C_O]^\circ$, $[C_R]^\circ$: concentrations of the oxidized and reduced ions in the bulk of the solution.

$[A_O]^\circ$, $[A_R]^\circ$: activities of the oxidized and reduced ions in the bulk of the solution.

f_O, f_R: activity coefficients of the oxidized and reduced ions in the solution.

K_O, K_R: constants concerning the diffusion of the oxidized and reduced ions.

If it is assumed that the electrode reaction and the diffusion are kept in an equilibrium at the electrode surface, I, $[A_O]$ and $[A_R]$ can be written as the function of V, $[A_O]^\circ$ and $[A_R]^\circ$ using the equations (3), (4) and (5). The expression of $[A_O]$ is obtained as the equations (6) and (6′) and that of $[A_R]$, as the equations (7) and (7′).

$$[A_O] = \frac{K_O'[A_O]^\circ(\kappa_2 E_2 + K_R') + K_R'[A_R]^\circ\kappa_2 E_2}{K_R'\kappa_1 E_1 + K_O'K_R' + K_O'\kappa_2 E_2} = \frac{[A_O]^\circ\left(\frac{1}{K_R'}\frac{\kappa_2 E_2}{\kappa_1 E_1} + \frac{1}{\kappa_1 E_1}\right) + \frac{1}{K_O'}[A_R]^\circ\frac{\kappa_2 E_2}{\kappa_1 E_1}}{\frac{1}{K_O'} + \frac{1}{\kappa_1 E_1} + \frac{1}{K_R'}\frac{\kappa_2 E_2}{\kappa_1 E_1}} \tag{6}$$

$$= \frac{[A_O]^\circ\left(\frac{1}{K_R'} + \frac{1}{\kappa_2 E_2}\right) + \frac{1}{K_O'}[A_R]^\circ}{\frac{1}{K_O'}\frac{\kappa_1 E_1}{\kappa_2 E_2} + \frac{1}{\kappa_2 E_2} + \frac{1}{K_R'}} \tag{6′}$$

$$[A_R] = \frac{K'_O[A_O]^\circ\kappa_1E_1 + K'_R[A_R]^\circ(\kappa_1E_1 + K'_O)}{K'_R\kappa_1E_1 + K'_OK'_R + K'_O\kappa_2E_2}$$

$$= \frac{\frac{1}{K'_R}[A_O]^\circ + [A_R]^\circ\left(\frac{1}{K'_O} + \frac{1}{\kappa_1E_1}\right)}{\frac{1}{K'_O} + \frac{1}{\kappa_1E_1} + \frac{1}{K'_R}\frac{\kappa_2E_2}{\kappa_1E_1}} \tag{7}$$

$$= \frac{\frac{1}{K'_R}[A_O]^\circ\frac{\kappa_1E_1}{\kappa_2E_2} + [A_R]^\circ\left(\frac{1}{K'_O}\frac{\kappa_1E_1}{\kappa_2E_2} + \frac{1}{\kappa_2E_2}\right)}{\frac{1}{K'_O}\frac{\kappa_1E_1}{\kappa_2E_2} + \frac{1}{\kappa_2E_2} + \frac{1}{K'_R}} \tag{7'}$$

Substituting the equations (6) and (7) or (6′) and (7′) for $[A_O]$ and $[A_R]$ in the equation (3), I is written as follows:

$$I = \frac{n\varepsilon K'_O K'_R([A_O]^\circ\kappa_1E_1 - [A_R]^\circ\kappa_2E_2)}{K'_R\kappa_1E_1 + K'_OK'_R + K'_O\kappa_2E_2}$$

$$= n\varepsilon\frac{[A_O]^\circ - [A_R]^\circ\frac{\kappa_2E_2}{\kappa_1E_1}}{\frac{1}{K'_O} + \frac{1}{\kappa_1E_1} + \frac{1}{K'_R}\frac{\kappa_2E_2}{\kappa_1E_1}} \tag{8}$$

$$= n\varepsilon\frac{[A_O]^\circ\frac{\kappa_1E_1}{\kappa_2E_2} - [A_R]^\circ}{\frac{1}{K'_O}\frac{\kappa_1E_1}{\kappa_2E_2} + \frac{1}{\kappa_2E_2} + \frac{1}{K'_R}}. \tag{8'}$$

Since $\frac{\kappa_2}{\kappa_1} = e^{-\Delta F_0/RT} = e^{-nV_0F/RT}$, where V_0 symbolizes the normal oxidation-reduction potential, the following relation is obtained.

$$\frac{\kappa_2E_2}{\kappa_1E_1} = e^{n(V-V_0)F/RT}$$

Now, the assumption that the oxidation-reduction reaction concerned is controlled completely by the diffusion will be introduced. When the rate of reduction of the oxidized form at the electrode surface is extremely large compared with the rate of diffusion of the oxidized form, that is, $\frac{1}{\kappa_1E_1}$ is negligible compared with $\frac{1}{K'_O}$ and when the rate of oxidation of the reduced form at the electrode surface is extremely large compared with the rate of diffusion of the reduced form, that is, $\frac{1}{\kappa_2E_2}$ is negligible compared with $\frac{1}{K'_R}$, the same equation for current, the

equation (9), can be derived in both cases, but from the equation (8) in the former case and from the equation (8′) in the latter case.

$$I = n\,\varepsilon\,K'_O K'_R\,\frac{[A_O]^\circ - [A_R]^\circ e^{n(V-V_0)F/RT}}{K'_R + K'_O\,e^{n(V-V_0)F/RT}} \tag{9}$$

Further, the equation (10) is obtained by rearranging the equation (9).

$$e^{n(V-V_0)F/RT} = \frac{K'_R}{K'_O}\,\frac{n\,\varepsilon\,K'_O[A_O]^\circ - I}{I + n\,\varepsilon\,K'_R[A_R]^\circ} \tag{10}$$

The equations (9) and (10) express the relation of the electrode potential and the current, which are nothing but the formulae of the current-voltage curve in polarography.

The limiting case when the difference of V and V_0 is large should be considered. There are two cases, of which the one occurs when V is much more positive than V_0, and the other occurs when V is much more negative than V_0. In the first case $e^{n(V-V_0)F/RT}$, that is, $\frac{\kappa_2 E_2}{\kappa_1 E_1}$ becomes large and then $\frac{\kappa_1 E_1}{\kappa_2 E_2}$ becomes small; in other words $\frac{\kappa_1 E_1}{\kappa_2 E_2}$ is nearer to zero, the larger the difference of V ond V_0. Then, for the equation for the current, the equation (11) is obtained from the equation (8) or (8′) using the assumption of the diffusion-control:

$$I = -n\,\varepsilon\,K'_R[A_R]^\circ = -n\,\varepsilon\,K_R[C_R]^\circ \equiv (I_d)_R\,. \tag{11}$$

This represents nothing but the diffusion current for the anodic wave. Moreover it proves from the equation (7) or (7′) that $[A_R]$ is equal to zero in this condition.

In the second case, on the other hand, $e^{n(V-V_0)F/RT}$, that is, $\frac{\kappa_2 E_2}{\kappa_1 E_1}$ comes nearer to zero and then the equation for current is derived by the same manner as the equation (11).

$$I = n\,\varepsilon\,K'_O[A_O]^\circ \doteqdot n\,\varepsilon\,K_O[C_O]^\circ \equiv (I_d)_O\,. \tag{12}$$

This expresses the diffusion current for the cathodic wave. In this case it proves from the equation (6) or (6′) that $[A_O]$ is equal or nearly equal to zero.

From the equations (11), (12) and (10), is obtained

$$e^{n(V-V_0)F/RT} = \frac{K'_R}{K'_O}\,\frac{(I_d)_O - I}{I - (I_d)_R}\,, \tag{13}$$

which is the formula of current-voltage curve employed most generally

in polarography.

When $\kappa_1 E_1$ and $\kappa_2 E_2$ have a magnitude which is nearly the same and when they are both extremely large compared with K'_O and K'_R, from the equations (6) and (7) or (6′) and (7′) is obtained

$$e^{n(V-V_0)F/RT} = \frac{[A_O]}{[A_R]}. \tag{14}$$

This is the same as the Nernst's equation which expresses the static electrode potential.

Finally the following must be emphasized. In the direct derivation of the formula of the current-voltage curve in polarography that is based on chemical kinetics, two fundamental assumptions are taken: the one is that the electrode reaction at the electrode surface and the diffusion are strictly kept in an equilibrium, and the other is that the rates of all of the possible intermediate steps in the oxidation-reduction reaction are so much more rapid than the diffusion that the current at any point on the polarographic wave is diffusion-controlled. However, to attain the equations (9) and (10), it is not absolutely necessary that both rates of the electrode reactions of the oxidized and the reduced forms are much larger than the rates of diffusions of corresponding forms. In other words, if the rate of diffusion of oxidized form can be rate-determining, it makes no difference whether on the reduced form the rate of electrode reaction is larger than the rate of diffusion or smaller. The converse is also true. This is obvious by the fact that the same equation, the equation (9), can be derived from the equations (8) and (8′) in the corresponding cases. To obtain the Nernst's equation, on the other hand, it is absolutely necessary that both rates of the electrode reactions of the oxidized and the reduced forms have the magnitude nearly the same as each other, and they are much larger than the rates of the diffusions of the corresponding forms.

Deposition of metals from simple (or hydrated) metal ions. In this case the following reaction should be considered instead of the equation (1).

$$\mathrm{M}^{n+} + n\ominus \rightleftarrows \mathrm{M(Hg)}, \tag{15}$$

where M^{n+} symbolizes the simple (or hydrated) metal ion of n valences and M(Hg), amalgam of the metal deposited by the electrode reaction.

If the activities of the metal ion and the metal in amalgam at the electrode surface are represented by $[M^+]$ and $[M]$ respectively and the activities of the metal ion in body of solution and the metal in body of the dropping mercury electrode, by $[M^+]^\circ$ and $[M]^\circ$, $[M^+]$, $[M]$, $[M^+]^\circ$ and $[M]^\circ$ correspond to $[A_O]$, $[A_R]$, $[A_O]^\circ$ and $[A_R]^\circ$ respectively in the equations (2) to (14) described above. Therefore, the equations for the

deposition of metal can be obtained by substituting $[M^+]$, $[M]$, $[M^+]^\circ$ and $[M]^\circ$ for $[A_o]$, $[A_R]$, $[A_o]^\circ$ and $[A_R]^\circ$ respectively in those equations. In this case K'_R signifies a constant concerning the diffusion of the metal in mercury.

If the case when pure mercury is employed for the dropping mercury electrode is taken up, the activity of the metal in body of the dropping mercury electrode, $[M]^\circ$, can be regarded as zero, and hence $[A_R]^\circ$ can be substituted by zero. As the result of this substitution, the following equations are obtained for the formula of the current-voltage curve in polarography, corresponding to the equations (9) and (10).

$$I = \frac{n\varepsilon K'_o K'_R [M^+]^\circ}{K'_R + K'_o e^{n(V-V_0)F/RT}} \tag{16}$$

$$e^{n(V-V_0)F/RT} = \frac{K'_R}{K'_o} \frac{n\varepsilon K'_o[M^+]^\circ - I}{I} \tag{17}$$

As to the equation for diffusion current, in the case of the oxidation-reduction reaction two equations are obtained; the one, for cathodic, and the other for anodic. In this case, however, for the diffusion current only one equation, the equation (18), is given, since $(I_d)_R$, that is, the equation (11) becomes zero because $[M]^\circ$ which corresponds to $[A_R]^\circ$ in the equation (11) is zero.

$$I = n\varepsilon K'_o[M^+]^\circ \equiv I_d . \tag{18}$$

Summary.

The formula of the current-voltage curve in polarography has been derived from the view-point of chemical kinetics and independently of the Nernst's equation. As a result, it has been clearly shown that if the condition of the diffusion-control is fulfilled the formula which had been derived from the Nernst's equation has been obtained in the various types of the electrode reaction. Of these types both the oxidation-reduction of the simple (or hydrated) metal ions and the deposition of the metals from the simple (or hydrated) metal ions have been dealt with in the present paper.

It is also suggested that the "irreversible wave" which cannot be treated theoretically based on the Nernst's equation can possibly be dealt with by the similar derivation as mentioned here.

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Étude biochimique sur les éléments des terres rares. II. Sur l'absorption du néodyme dans le corps animal.

Par Réinosouké HARA

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Introduction. Jusqu'ici, par l'administration *pérorale*, l'absorption du sel des terres rares a été faite par C. Bachaem,[1] G. Baehr et H. Wessler,[2] R. Niccolini,[3] et l'injection intramusclaire par G. Baehr. et Wessler,[4] M. Ajazzi-Manchini,[5] P. Niccolini,[6] G. Guidi.[7] Ils ont demontré que l'absorption des sels du néodyme dans le corps animal est trés peu considérable. Je l'ai étudiée par l'analyse spectroscopique à rayons X. Les sels employés sont le chlorure du néodyme soluble, et l'oxalate du néodyme insoluble.

Expérience

Dans le cas de l'administration pérorale. En employant trois rats, j'ai donné à chaque animal 0.05 g de chlorure du néodyme ou d'oxalate du néodyme par jour, pendant cinq jours. Après cela, ces rats ont été tués et on a incinéré les viscères et procédé à l'analyse spectroscopique à rayons X. Le résultat de l'analyse est montré ci-dessous.

(a) *Dans le cas de chlorure du néodyme.*

Table 1.

		NdLα_1	NdLα_2	NdLβ_1	PrLα_1	PrLβ_1			NdLα_1	NdLα_2	NdLβ_1	PrLα_1	Pr Lβ_1
	1	⫴	+	+	±	±		1	⫲	±	±		
foie	2	⫲	+	+			pancréas	2	+	±			
	3	⫲	+	+	±			3	+				
	1	⫲	+	+	±			1	⫲	±	+	±	
rate	2	⫲	+	+			poumon	2	+	±			
	3	⫴	+	+	±	±		3	+	±			
	1	⫫	±	+				1	+				
rein	2	+	±				coeur	2	+	±			
	3	+						3	+	±			

⫴ — très fort, ⫲ — assez fort, + — faible, ± — très faible
"1, 2, 3," indiquent les numéros des animaux.

(1) C. Baechaem, *Arch. internat. Pharmacodynamie*, **17** (1907), 381.
(2) G. Baehr et H. Wessler, *Arch. internat. Med.*, **2** (1908), 517.
(3) P. Niccolini, *Arch. internat. Pharmacodynamie*, **37** (1930), 222; **40** (1931), 262.
(4) G. Baehr et H. Wessler, *Arch. internat. Med.*, **2** (1908), 526.
(5) M. Ajazzi-Manchini, *Arch. di, Fisiol.*, **25** (1927), 43.
(6) P. Niccolini, *Arch. internat. Pharmacodynamie.*, **37** (1930), 221; **40** (1931), 261.
(7) G. Guidi, *Arch. interuat. Pharmacodynamie.*, **37** (1930), 344; **39** (1930), 1.

(b) *Dans le cas de l'oxalate du néodyme.*

Table 2.

		Nd $L\alpha_1$			Nd $L\alpha_1$			Nd $L\alpha_1$
foie	1	±	rein	1		poumon	1	±
	2	±		2	±		2	±
	3			3			3	±
rate	1		pancréas	1		coeur	1	±
	2	±		2			2	±
	3	±		3	±		3	

Par l'administration *pérorale* du sel soluble, dans le foie et la rate, la ligne Nd $L\alpha_1$ est très forte ou assez forte à peu près sans exception, et on a trouvé en outre les lignes Nd $L\alpha_2$, Nd $L\beta_2$, Pr $L\alpha_1$, Pr $L\alpha_2$. Aux autres viscères, on a trouvé les lignes Nd $L\alpha_1$, Nd $L\alpha_2$. Cést peut-être que le sel a changé en forme insoluble dans le sang, et s'est fixé dans chaque viscère. Par l'administration *pérorale* du sel insoluble, on a trouvé la ligne Nd $L\alpha_1$ très faible dans chaque viscère.

Dans le cas de l'injection intramusclaire. En employant trois rats, j'ai fait l'injection intramusclaire 0.4 c.c. de la solution du chlorure du néodyme à 0.5% à chaque animal, au

Table 3.

		Nd $L\alpha_1$	Nd $L\alpha_2$	Nd $L\beta_1$	Pr $L\alpha_1$	Pr $L\beta_1$			Nd $L\alpha_1$	Nd $L\alpha_2$	Nd $L\beta_1$	Pr $L\alpha_1$	Pr $L\alpha_1$
foie	1	++	±	+			poumon	1	+	+			
	2	++	±	+				2	++	±	+		
	3	++	+					3	+	+			
rate	1	+++	+	+	±	±	coeur	1	+	+	+		
	2	++	±	+	±			2	++	±		±	
	3	++	±	+	±			3	+	+			
pancréas	1	+					estomac, intestin et leurs contenus	1	+				
	2	+						2					
	3	+	+					3					
rein	1	+	±				endroit de l'injection	1	+++	+	+	±	±
	2	+						2	+++	+	+	±	±
	3	+	±					3	+++	+	+	±	±

(8) G. Guidi, *Arch. internat. Pharmcodynamie.*, **37** (1930), 318.

dos. Cinq jours après, je les a tués et incineré les viscères, et fait l'analyse spectroscopique à rayons X. D'après G. Guidi,[8] le dose mortelle du chlorure du néodyme aux rats est 0.15–0.25 g./kg.

Le resultat de l'analyse est montré dans la table 3.

Dans le cas d'injection intramusclaire, la ligne Nd L_{α_1} dans l'endroit de l'injection est très forte sans exception, et on a trouvé les lignes Nd L_{α_2}, Nd L_{β_1}, Pr L_{α_1}, Pr L_{β_1}.

Conclusion, (1) Par l'administration *pérorale*, l'absorption du sel soluble (chlorure du néodyme) n'est pas remarquable. Et de même l'injection intraveine, le sel a été trouvé dans le foie et la rate. Aux autres viscères, on a trouvé le sel, c'est peut-être que le sel a changé en forme insoluble dans le sang, et s'est fixé dans chaque viscère.

(2) Par l'administration *pérorale*, le sel insoluble (oxalate du néodyme) est absorbé en petite quantité, c'est-à-dire, on a trouvé la ligne N_{α_1} très faible dans chans chaque viscère.

(3) En l'injection intramusclaire, l'absorption du chlorure du néodyme est très faible, la plus grande quantité est fixée dans l'endroit de l'injection. De même dans les autres cas, dans le foie et la rate, on a trouvé le sel. Dans les autres viscères, on a trouvé le sel sans exception car le sel est fixé dans le sang.

(4) Quand on donne au corps animal les sels du néodyme par diverses méthodes, l'absorption et l'évacuation sont très faibles car les sels changent à certaine forme insoluble dans l'organisme, et se déposent dans les viscères constitués en grande partie par le système réticulendothériale.

À la fin de ce compte-rendu, je dévoue mes reconnaissances plus profondes à M. le Prof. Kénjiro Kimura pour les instructions extrêmement soigneuses, à M. le Prof. Kéigi Misawa et à M. le Prof. adjoint Taro Kawamura pour les conseils favorables au sujet des expériences zoologiques et à M. M. les Profs. Taku Uémura, Eiji Ochiai, et Yosoji Ito et à M. le Prof. adjoint Kazuo Kuroda pour les suggestions pleines de pénétrations.

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Fungus and Enzyme.
Preparation of Aspergillus Oryzae Amylase

By Taichi HARADA.

(Received March 1, 1949).

The role of fungi in nature is of very great importance to our daily life.

Pathogenic fungi are those which cause a very annoying disease "Eczema" in the human body[1] and also plant diseases in plants. Non-pathogenic species are those which cause deterioration and destruction of timbers, textiles and foods, and those which produce many organic acids,[2] e. g. citric, lactic, gluconic, kojic, etc., arsine compounds, penicillin,[3] etc. and also those which produce many important enzymes, e. g. amylase, protease, tannase, invertase, etc. This is, of course, speaking in a broad sense of the difference between the pathogenic and the non-pathogenic species.

The amount of the enzymes produced by fungi depends upon the composition of culture medium in which they are grown. According to Takada[4] wheat bran increases the production of Asp. oryzae enzyme as much as two times as that of embryo of rice bud and five times as that of oil removed bean. In this connection the author refers to his previous paper[5] in which very important precautions of making Koji were pointed out.

In the preservation of enzyme in the Koji cake the cake may be dried to prevent bacterial infections or after crushing the cake loosely the enzyme may be extracted at once with cold water and then filtered and preserved in a manner suitable for the purpose for which the preparation is to be used. Sodium chloride at the saturation point gives the most satisfactory results as an antiseptic. This solution may be used for most purposes; oral administration, food preparation or for the desizing of textiles. The amylase prepared from the water extract by alcohol precipitation is not a pure single enzyme but a mixture of many enzymes.[6]

(1) T. Harada, This Bulletin, **9** (1934), 186, 192.

(2) Prescott and Dunn, "Industrial Microbiology", McGraw-Hill Book Co., New York (1940).

(3) Fleming, *Brtt. J. Exp. Path*, **10** (1929), 266; "Penicillin", Hekiso Soc., Kawade Book Store, Tokyo (1946); also Sumiki's "Penicillin", Sangyo Tosho Co., Tokyo (1946).

(4) *J. Brew. Asstn. Japan*, **22** (1944), 232; also Takada, Katsui and others, *J. Appl. Micology*, **2** (1948), 88.

(5) T. Harada, *Ind. Eng. Chem.*, **23** (1931), 1424.

(6) Inouye, "Useful Molds", Hokko Book Store, Tokyo (1948).

(7) T. Harada, *Ind. Eng. Chem. Anal. Ed.*, **3** (1931).

In the factory, in precipitating the enzyme with alcohol we often encounter great trouble when the Koji water extract becomes unusual in nature giving a colloidal (light) or a gummy (heavy) precipitate which can not be thoroughly dehydrated. In other words, it does not give a flocculent precipitate. When a gummy precipitate is obtained, it must be dissolved in water and then the precipitation must be carried out again. Such a colloidal or a gum-like precipitate could not be easily dried or if it is dried a dark lump may be obtained. In both cases not only is it very difficult to make it in powder form after drying, but the diastatic strength becomes greatly lost. Of course the trouble of gummy formation may be removed by cutting down the amount of alcohol but this decreases the enzyme. It was found that, in case the precipitate tends to become colloidal, an addition of a small amount of sodium phosphate, or potassium phosphate, or the like to the extract not only solves the trouble but also gives an increase of diastatic power to the preparation in its total strength. However, in the case of gummy formation the dibasic halogen compound will solve the trouble but it will decrease the total Lintner's value (L. V.). This is due to a destruction of the enzyme.

Experimental

Influence of Inorganic Salts upon Amylase Precipitation. Twenty five cubic centimeters of the water extract of Koji were mixed with a small amount of inorganic salts to which 75 c.c. of alcohol were added for the precipitation. It was mixed well, filtred, dried and finally the strength determined by the author's[7] method for L.V.

Table 1.

Salts (0.05 g.)	Yield (g.)	Tested L.V.	Total L.V.
$(NH_4)_2SO_4$	0.6370	3816	2431
KH_2PO_4	0.6211	4133	2567
$NaH_2 \cdot PO_4 \cdot H_2O$	0.6377	4133	2636
K_2HPO_4	0.6475	3933	2547
$Na_2HPO_4 \cdot 5H_2O$	0.6595	4033	2660
Alcohol alone	0.6361	3617	2301

Original total L.V. of 25 c.c. of the extract $(111 \times 25) = 2775$

Table 2.

Salts	(g.)	Yield (g.)	Tested L.V.	Total L.V.
$MgCl_2 \cdot 6H_2O$	0.05	0.6330	2875	1820
"	0.10	0.6635	2450	1626
"	0.20	0.6910	1500	1037
$CaCl_2 \cdot 2H_2O$	0.05	0.5710	3075	1756
"	0.10	0.6480	1825	1183
"	0.20	0.7680	1000	768
Alcohol alone		0.6190	3075	1903

Original total L.V. of 25 c.c. of the extract $(159 \times 25) = 3975$

Table 3.

Salts (0.10 g.)	Yield (g.)	Tested L.V.	Total L.V.
NaCl	0.6714	4775	3206
$NaHCO_3$	0.8250	3975	3280
$Na(NH_4)_2PO_4 \cdot 4H_2O$	0.6925	4450	3082
$MgSO_4 \cdot 7H_2O$	0.7580	4175	3165
$(NH_4)_2HPO_4$	0.6817	3773	2573
K_2HPO_4	0.6965	4175	2908
Alcohol alone	0.6905	3975	2745
Original total L.V. of 25 c.c. of the extract		(159×25)=3975	

Table 4.

Salts (g.)		Yield (g.)	Tested L.V.	Total L.V.
$MgCl_2 \cdot 6H_2O$	0.10	0.6765	5725	3873
"	0.20	0.7145	3975	2840
$CaCl_2 \cdot 2H_2O$	0.10	0.6625	5100	3379
"	0.20	0.8045	1525	1227
Alcohol alone		0.6560	6350	4166
Original total L.V. of 25 c.c. of the extract			(191×25)=4775	

Preparation of Dry Amylase without Alcohol for Oral Purpose. In preparing the amylase in tablet form for oral administration, the Koji extract was mixed with magnesium or calcium carbonate in the ratio of about three to one and placed in a large flat container and while slowly mixing it, it was dried by means of a dry air current at low temperature (30-35°C). L.V. of the mixture was obtained up to 750. This amylase-carbonate mixture may be easily made into tablets.

Summary

1. The influence of inorganic salts upon the precipitation of amylase with alcohol under given conditions has been studied.

2. The preparation of dry amylase without alcohol for oral purpose has been described.

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Studies on Foams. III. The Foam Formation of Some Dyestuff Solutions.

By Masayuki NAKAGAKI.

(Received March 14, 1949)

It has been reported, in the preceding paper[1], that the abilities of foam formation of some mixtures of organic liquids are correlated with the volume contraction on admixture, and the latter may probably be related with the amphipathy of solute molecules. That is to say, the balance between the polar and non-polar portion of the molecule has been considered as an essential factor governing the foam formation. If this might be true also of dyestuff solutions known as "micelle colloids", some connection may be found among the foam formation, surface activity, and molecular structure of the dyestuffs.

The foam formation and the surface tension have been measured on the aqueous solutions of congo red and five triphenyl methane dyes: methyl violet 5 B, brilliant green, malachite green (crystallized), crystal violet (chemical pure), and Hofmann's violet. E. Merck's dyes have been used without further purification, excepting congo red, which has been purified by the sodium acetate-alcohol method. Dyestuff solutions were preserved for 1 to 36 weeks as the solution of 1g./l., and diluted just before the measurements.

The foam formation was measured by the shaking test; 20 c.c. of the solution was taken into a test tube, 40 c.c. in capacity, and 1.8cm. in diameter and provided with a glass stopper. This was shaken up and down for 30 seconds at the rate of 3 times a second and at the amplitude of 25 cm. The maximum foam zone height A_m (cm.) is considered as a measure of the foaminess of the solution. The foam strength τ(sec./cm.) is defined as the reciprocal of the collapsing velocity of foam within the initial thirty seconds. Thus,

$$\left.\begin{aligned} &\tau = 30/(A_m - A_{30}) && \text{for } t > 30, \\ \text{or}\quad &\tau = t/A_m && \text{for } t < 30, \end{aligned}\right\}$$

where A_{30} is the foam height after 30 seconds from the instant of maximum foam height, and t is the foam duration in seconds. The foaming ability (F) of the solution (Fig. 1) is defined as the products of foaminess (A_m) and foam strength (τ). The surface tension was measured by du Nouy's tensiometer. The viscosities of these solutions were also measured, and concluded that they have no appreciable effects on the foam formation, since the values relative to water are 1.00 to 1.02.

(1) The second paper, This Bulletin, **22** (1949), 21.

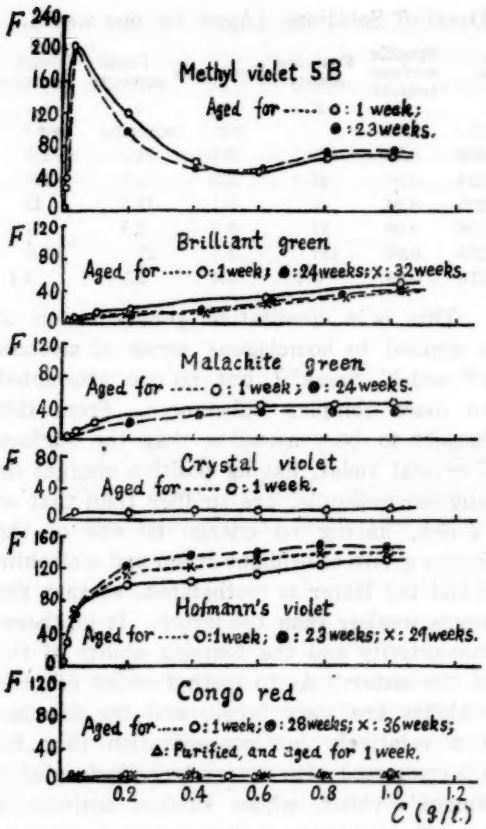
F
Methyl violet 5B
Aged for ····· ○: 1 week;
●: 23 weeks.
Brilliant green
Aged for ····· ○: 1 week; ●: 24 weeks; ×: 32 weeks.
Malachite green
Aged for ····· ○: 1 week; ●: 24 weeks.
Crystal violet
Aged for ····· ○: 1 week.
Hofmann's violet
Aged for ····· ○: 1 week; ●: 23 weeks; ×: 29 weeks.
Congo red
Aged for ····· ○: 1 week; ●: 28 weeks; ×: 36 weeks;
△: Purified, and aged for 1 week.
C (g/l.)

Fig. 1. Foaming Abilities.

The effect of aging of the solution on the foam formation is not serious, and the change of foam formation by the purification of dye is also insignificant.

It is shown in Table 1 that the solution of large foaming ability has small surface tension excepting Hofmann's violet. Such a relation may be interpreted as follows. The ion of crystal violet, e. g., undergoes quantum mechanical resonance among three equivalent structures, and in consequence, the molecule takes the shape of a regular triangle, and three nitrogen atoms equally have 1/3 of the positive charge as the average. This was stated by E. Hückel[2] and G. N. Lewis and M. Calvin[3], and the symmetrical structure interpreted satisfactorily the absorption spectra obtained by G.E.K. Branch and M. Calvin.[4] On the other hand, malachite green or brilliant green has a phenyl radical which lacks the amino group, and this part of the molecule shows distinct hydrophobic character. Then, the molecule having both polar and non-polar parts in the molecule was named by G. S. Hartley[5] as "amphipathic" molecule. Such a molecule is adsorbed to the surface of the solution, turning the polar part to water and the non-polar part to air, and lowers the surface tension and increases the foam formation; the degree of which is greater when the hydrophobic character of the non-polar part is more distinct or the hydrophilic character

(2) E. Hückel, *Z. Elektrochem.*, **43** (1937), 827.

(3) G. N. Lewis and M. Calvin, *Chem. Rev.*, **25** (1939), 273.

(4) G.E.K. Branch and M. Calvin, "The Theory of Organic Chemistry," pp. 179, New York (1941).

(5) G. S. Hartley, "Aqueous Solutions of Paraffin-Chain Salts", Chapter 7, Paris (1936).

Table 1. Properties of Dyestuff Solutions. (Aged for one week).

Dyestuff	Concentration		Specific surface tension	Foaming ability	Foaminess	Foam strength	Foam duration
				F	A_m	τ	t
	(g./l.)	(mol/l.)	γ/γ_w		(cm.)	(sec./cm.)	(sec.)
Methyl violet 5B	1.01	0.00209	0.86	71	5.1	14	>200
Brilliant green	1.03	0.00214	0.90	47	3.7	12.7	>200
Malachite green	0.827	0.00227	0.94	42	3.1	13.5	42
Crystal violet	0.796	0.00196	0.96	11	8.9	2.8	11
Hofmann's violet	0.988	0.00235	0.96	187	4.7	27	>200
Congo red	1.52	0.00218	0.99	3.4	1.4	2.5	3.4

of the polar part is weaker. This is a qualitative generalization of Traube's rule, which has been applied to homologous series of alcohols and fatty acids by O. Bartsch[6] and T. Sasaki[7], but no one attempted hitherto to apply this idea to more complex substances. From this standpoint, however, it is thought to be reasonable that the surface activity and foaming ability of crystal violet, having positive charges on every apex of the regular triangular melecule, are smaller than that of brilliant green or malachite green, having no charge on one of the apexes. Then, comparing the amino group of brilliant green and malachite green, the former is ethylated and the latter is methylated, so that the hydrophilic nature of the former is weaker than the latter. It is, therefore, reasonable that the surface activity and the foaming ability of the former is greater than that of the latter. As to methyl violet 5B, the surface activity and foaming ability are very large, and the foaming ability becomes maximum at a relatively low concentration (Fig. 1), since one of the amino groups is connected with a very large hydrophobic group. One exception is Hofmann's violet, whose surface activity is small while the foaming ability is large. This is thought to be due to the strong interaction between amino groups which are only partially ethylated.

The sequence of the foaming ability of triphenyl methane dye solutions is thus interpreted with their structural formulae. Since the dominant factor is the properties of the molecule itself, it is supposed that the single molecule is mainly concerned with the foam formation and micelle plays no significant role.

Summary

The foam formations of the aqueous solutions of methyl violet 5B, brilliant green, malachite green, crystel violet, Hofmann's violet and congo red have been measured. The foaming ability, that is the product of foaminess and foam strength, is considered as the measure of the

(6) O. Bartsch, *Kolloid-Z.*, **38** (1926), 177.
(7) T. Sasaki, This Bulletin, **13** (1938), 517.

foam formation. This scarcely changes by the long period's storage of the solution. The foaming ability of them is nearly parallel with the surface activity. This is interpreted with structural formnlae of dyestuffs.

The author wishes to express his hearty thanks to Professor Jitsusaburo Sameshima for his kind encouragement.

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On the Equilibrium of the Radioactive Elements in the Hydrosphere. VI.[1] Radioactivity Measurement of Natural Waters with the Geiger-Müller Counter.

By Kazuo KURODA.

(Received March, 14, 1949.)

The radioactivity of a large number of natural waters in Japan has been mainly measured with the fontactoscopes, such as Schmidt-type, I.M.-type etc. The radioactivity measurement of natural waters with the Geiger-Müller counter was recently tried for the first time by the present author, and the results are reported in this paper.

Experimental Part. (1) Apparatus. A Geiger-Müller counter of Nippon-Denki Co. was used. The thickness of aluminum wall of the counter tube was about 0.1 mm. and the background was about 30 per minute. (2) Radioactivity measurement. The water sample (about 100 c.c.) was placed near the counter-tube (about 2 cm. from the wall of the counter-tube), and the number of counts per minute was recorded.

Results. (1) Relation between the number of counts and the radon content of natural waters. The results of radioactivity measurement of the radioactive springs of Misasa, Tottori Prefecture are shown in Table 1.

Table 1. Relation between the number of counts and the radon content of mineral water of Misasa.

Name	Radon Content (10^{-10} Curie/Litre)	Number of Counts (Counts/Minute)
Yamada-ku, Kyodo-yu, Nomi-yu	1044	19.5
OTR (Ohasi, Tennen-buro-Reisen)	263	19

(1) Previous Paper V. Kazuo Karoda, This Bulletin, **22** (1949), 149.

Okayama-University	59.5	5
Hanaya	741	8.5
Moto-Aburaya	142.5	3
Eirakuan		3.5
Akasakiya	85	0
Matsubara	111.1	7
Yakuba-no-yu	424	5.5
Gunze-no-yu	1021	15.5
Tap water		5.5
Distilled water	0	2

The number of counts is almost proportional to the radon content of natural water, but the natural waters containing thorium emanation show exceptionally large values of counts, as in the case of spring OTR of Misasa. The sensitivity of radioactivity measurement with the counter was considerably low compared with that of the fontactoscopes. Thus the radioactivity of the springs containing less than 10 Mache-unit of radon could not be detected with the counter.

(2) Increase of β and γ radioactivity of fresh mineral waters. It was reported in the previous paper,[2] that the intensity of β and γ radioactivity of fresh mineral water is usually very low, and it increases after the mineral water has issued, and a constant value is obtained within about three hours. It was explained in the previous paper to be due to the increase of the decay products of radon after the mineral waters have issued. This phenomenon was also observed in the case of the radioactivity measurement with the Geiger-Müller counter. The results are shown in Table 2.

Table 2. Increase of β and γ Radioactivity of Fresh Mineral Waters.

Name of the Springs	Time elapsed after the Springs issued (Minutes)	Number of Counts per Minute
Yamada-ku, Kyodo-yu Nomi-yu	15	2.5
	33	4
	63	15.5
	160	20
Yamada-ku, Kyodo-yu (Stagnant Water)	25	12
	73	17
	275	16
Seito-kan, Nomi-yu	22	9
	64	7
	165	17
	238	13
Gunze-no-yu	22	1
	60	15
	117	15
	180	14

(2) Kazuo Kuroda and Yuji Yokoyama, This Bulletin, **21** (1948), 52.

(3) Measurement of radioactivity due to radium with the Geiger-Müller counter. The radioactivity of mineral waters containing radium was measured with the Geiger-Müller counter, several months or several years after the sample was taken. The radioactivity of mineral waters containing high amounts of radium ($n\times10^{-12}$ to $n\times10^{-10}$ g. radium per litre) could not be detected with the Geiger-Müller counter, and it was concluded that it is impossible to estimate the radium content of natural waters directly with the counter.

(4) Radioactivity of Sinter Deposits. The radioactivity of sinter deposits of the thermal springs of Misasa and the mineral springs of Masutomi was measured with the Geiger-Müller counter. Some sinter deposits of Misasa showed considerably strong radioactivity, which is almost equal to that of monazite. This radioactivity is considered to be due to radium and mesothorium in the sinter deposits.

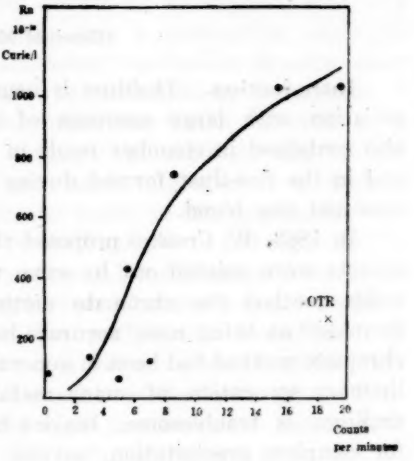

Table. 1. Relation between the number of counts and the radon content of mineral waters of Misasa.

Summary

The radioactivity of the natural water was measured with the Geiger-Müller counter. The relationship between the radon content of the thermal springs of Misasa and the number of counts was studied. The increase of β and γ radioactivity of fresh mineral waters was observed.

The author expresses his hearty tanks to Professor Kenjiro Kimura for his kind guidance. The cost of this research was defrayed from the Scientific Research Encouragement Grant from the Department of Education, to which the author's thanks are due.

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New Method of Determination of Thallium with Cobalt Hexammine Trichloride.

By Yukio MURAKAMI.

(Received March, 31, 1949.)

Introduction. Thallium is usually found in small quantities in association with large amounts of alkali metals and other elements and also contained in chamber muds in plants commercializing sulfuric acid, and in the flue-dust formed during the calcination of iron pyrites, lead ores and zinc blend.

In 1863, W. Crookes proposed the iodide method[1]. However, several defects were pointed out by some workers,[2] and as a substitute for the iodide method the chromate method was recommended by Moser and Brunkle[3] as being most accurate in the determination of thallium. This chromate method had been in general use for a long time, but as the preliminary separation of many metals that are commonly present with thallium is troublesome, besides having to allow a little over 12 hours for complete precipitation, several other methods were in continuous study by many workers. No one proposed method yet has been approved for wide use. On the other hand, the application of the organic reagents, such as thionalide,[4] mercapto-benzothiazol[5] and thiourea[6], to the determination of thallium has been recommended because of their good sensitivity. However, when not more than 10 mg. in a 50 gr. sample of flue-dust and chamber muds containing a large quantity of common metals is to be determined, none of the so-far suggested methods is qualified as a sufficient answer to the problem.

Among the above mentioned methods, the thionalide method is the most sensitive, though it does not show specific reaction to the thallium (Mehrdeutigkeit) and a rough estimation as to the test sample is required before the thionalate formation can be made. In a case of minute

(1) W. Crookes, *Chem. News*, **7** (1863), 133; *J. Chem. Soc.*, **17** (1864), 115; G. Werther, *Z. anal. Chem.*, **3** (1864), 1; H. Baubigny, *Compt. rend.*, **113** (1891), 544; W. Lepper, *Z. anal. Chem.*, **79** (1930), 323.

(2) F. Mach and W. Lepper, *Z. anal. Chem.*, **68** (1926) 40; F. Cuta, Collection Czechoslovak. Chemical Communications, **7** (1935), 33; W. Lepper, *Z. anal. Chem.*, **79** (1930), 323, H. Baubigny, *Chem. News*, **64** (1891), 239.

(3) L. Moser and A. Brukle, *Monatsh.*, **47** (1926), 709; J. Bondanar and A. Terenyi, *Z. anal. Chem.*, **69** (1926), 32; A. Cernotzky, *Z. anal. Chem.*, **93** (1933), 349.

(4) R. Berg and Fahrenkamp, *Z. anal. Chem.*, **109** (1937) 305; R. Berg and W. Roebling, *Angew. Chem.*, **48** (1935), 430.

(5) G. Spacu and K. Kuras, *Z. anal. Chem.*, **109** (1936), 90.

(6) C. Mahr and O. Ohle, *Z. anal. Chem.*, **115** (1939), 256.

thallium determination in such samples as in flue-dust and chamber muds occurring with an abundance of many other metals, it is very difficult and impractical according to the author's experience. The thiourea method with which perchloric acid is required has its defects, due to the formation of an elastic compound resembling india-rubber when confronted with specially large quantities of copper, zinc and lead. Moreover the Tl-thiourea complex has a perceptible solubility even in ice cold water. It is an unsatisfactory method for determination of such minute amounts of thallium as was attempted by the author.

In the face of these difficulties the author has taken interest in a suggestive report by Spacu *et al*[7] stating that thallium can be determined as $Co(NH_3)_6(TlCl_6)$ which is practically insoluble in a slightly acidic medium. In this paper, though the result of the gravimetric determination of considerable amounts of thallium (80 to 100 mg.) only in a pure salt solution is reported, the experimental conditions are not given in details and examination of the interference of many metals, which is of the most practical importance, was not performed. However it is easy to suppose that the determination of a minute quantity of thallium in such samples with accurate results is possible as the factor for thallium in $Co(NH_3)_6(TlCl_6)$ is the least (0.35346) of all factors in the gravimetric method.

This investigation is based upon the above report and was undertaken for the following purposes: (a) To determine the interference of other metals, if any. (b) To note the experimental conditions and to determine as little as 5 to 10 mg. of thallium. (c) To determine whether it is applicable to the determination of thallium in flue-dust and chamber muds.

Experimental.

(a) Reagent used. *Thallium standard solution.* Thallium sulfate, Merck reagent (For Analysis), was recrystallized twice and heated to about 400°C in an electric furnace. The standard solution was prepared by dissolving the salt thus obtained.

Cobalt hexammine trichloride $Co(NH_3)_6Cl_3$ (for the sake of convenience, called luteo salt hereafter). Luteo salt is easily prepared from cobalt chloride, ammonium chloride, silver nitrate and ammonia according to Biltz,[8] and recrystallized from saturated solution by the addition of hydrochloric acid. The saturated solution, the concentration of which is about 20 M at room temperature, was used. It is desirable that any contaminable matter be filtered off before using and to preserve it in amber-colored bottle as decomposition will take place with exposure to light.

(b) Addition of ammonium chloride. The original report stated the greater possibility of success by making a precipitation under 2.5N hydrochloric acid solution. The suitable acid concentration in this case was tested as shown in Table 1.

These results indicated that it may be safer to use a 2.5N acid solution as previously reported. However, the lower results were obtained due to incomplete pre-

(7) G. Spacu and A. Pope *Z. anal. Chem.*, **120** (1940), 322.

(8) H. Biltz, „Übungsbeispiele aus der unorg. Experimentalchemie", S. 165, Leipzig (1913).

Table 1. Acid Concentration

Acid Concentration	$Co(NH_3)_6TlCl_6$ (g.)	Tl-found (g.)	Tl-present (g.)	Difference (g.)	Remarks
0.33N HCl	0.0284	0.01004	0.01002	+0.00002	added some NH_4Cl
0.66N HCl	0.0288	0.01018	0,01002	+0.00016	instantly pptd.
1.32N HCl	0.0283	0.01000	0.01002	−0.00002	,,
1.98N HCl	0.0238	0.01018	0.01002	+0.00016	
3.30N HCl	0.0298	0.01060	0.01002	+0.00058	luteo salt pptd.
0.82N HNO_3	0.0265	0.00937	0.00970	−0.00033	
2.26N HNO_3	0.0290	0.01025	0.00970	+0.00055	
3.86N HNO_3	0.0064	0.00227	0.00970	−0.00743	precipitate dissolved
5.46N HNO_3	0.0005	0.00047	0.00970	−0.00923	,,
1.02N H_2SO_4	—	—	0.00970	−0.00970	not precipitated
2.46N H_2SO_4	0.0237	0.00837	0.00970	−0.00133	
4.26N H_2SO_4	0.0168	0.00594	0.00970	−0.00376	
7.86N H_2SO_4	—	—	0.00970	−0.00970	,,

cipitation as sometimes happen especially with sulfuric acid or nitric acid solution owing to the amount of chloride ion present being insufficient to form $TlCl_6'''$. But this difficulty is easily removed by the addition of ammonium chloride as shown in Table 2 and the range of acid concentration is safely extended to about 3N. Moreover the determination can be made in any kind of acid medium.

It was also found that the addition of about 0.5 g. ammonium chloride is responsible for the perfect change of thallium ion into $TlCl_6'''$. Furthermore it makes possible the determination of thallium as slight as 5 mg. gravimetrically indeterminable by any other method up to date. Thallium is not easily oxidized with nitric acid alone, but its complete oxidation can be effected in the presence of any chloride ion. As there is a tendency that hydrolysis of thallic nitrate or sulfate will occur, especially when hot, the formation of $TlCl_6'''$ is sometimes difficult. But ammonium chloride removes also these difficulties. Consequently the addition of ammonium chloride newly recommended is remarkably useful in this instance, especially with sulfuric acid solution most commonly used.

Table 2. Effectiveness of the Addition of NH_4Cl

Acid Concentration	HCl (ml.)	NH_4Cl (g.)	Tl-complex (g.)	Tl-found (g.)	Tl-present (g.)	Difference (g.)
2.0N H_2SO_4	15	0.5	0.0324	0.01145	0.01149	−0.00004
2.0N H_2SO_4	15	none	0.0311	0.01099	0.01149	−0.00050
2.0N H_2SO_4	none	0.5	0.0325	0.01149	0.01149	−0.00000
2.0N H_2SO_4	none	none	0.0229	0.00081	0.01149	−0.01068
1.9N HNO_3	15	0.5	0.0326	0.01152	0.01149	+0.00003
1.9N HNO_3	15	none	0.0235	0.01007	0.01149	−0.00142
1.9N HNO_3	none	0.5	0.0308	0.01089	0.01149	−0.00060
1.9N HNO_3	none	none	0.0237	0.00105	0.01149	−0.01044
2.5N H_3PO_4	none	0.5	0.0301	0.01064	0.01032	+0.00032
2.5N H_2SO_4	5	0.5	0.0304	0.01075	0.01032	+0.00043
2.5N H_2SO_4	5	none	0.0232	0.00997	0.01032	−0.00035

(c) **General procedure.** Oxidation can be easily carried out with some particles of potassium chlorate and about 0.5 g. ammonium chloride added to the boiling solution. Boiling is continued until the volume is reduced to one half, and then it is allowed to stand at room temperature. Thus the solution is ready for the precipitation. If luteo salt solution is added, a brown orange precipitate forms instantanously. When the temperature

of the solution exceeds 50°C, thallic oxide may be precipitated, the formation of which should be avoided.

Twice the amount of luteo salt solution should be added, though theoretically it is sufficient to add 1.3 times the amount of thallium expected in the solution. The precipitate is decanted with 2% HCl solution and transferred to a glass filter G4. Washing must be continued until the washing solution becomes colorless, usually requiring more than 200 ml. for 10 mg. thallium. The precipitate then is washed three times with 5 ml. each of 95% alcohol followed by three times with ether. The precipitate is then dried at 105°C from a half to an hour, and transferred in desicator to cool at room temperature. Thallium now is weighed as $Co(NH_3)_6(TlC_6)$ (F = 0.35346).

The result agrees with the true value within the errors allowed as shown in Table 3. However deviation may be caused, maximum deviation of results was found less than 1.5% of thallium. The remarkable characteristics of this method is that a minute amount of thallium can be determined with fairly good results by the addition of ammonium chloride without any hazardous operation.

Table 3. Accuracy

Tl-present (g.)	$Co(NH_3)_6(TlCl_6)$ (g.)	Tl-found (g.)	Difference (g.)	Error %
0.00574	0.0163	0.00576	+0.00002	+0.35
0.00484	0.0135	0.00480	−0.00004	−0.82
0.00968	0.0275	0.00972	+0.00004	+0.41
0.00968	0.0270	0.00954	−0.00014	−1.4
0.00968	0.0269	0.00950	−0.00018	−1.8
0.01148	0.0321	0.01149	+0.00001	+0.00
0.01937	0.0551	0.01949	+0.00010	+0.51
0.01937	0.0543	0.01919	−0.00018	−0.92
0.01937	0.0546	0.01930	−0.00007	−0.35
0.01937	0.0547	0.01940	+0.00003	+0.16
0.02903	0.0822	0.02905	+0.00002	+0.07
0.03980	0.1126	0.03973	−0.00007	−0.18

Proposed Method. For practical application, it is necessary to get sufficient knowledge on the behavior of various other ions on luteo salt. However as this proposed method isolates some ions precipitating with luteo salt, for the sake of convenience, this new method will be discussed before the interference is discussed.

(*a*) *Proposed Method.* Finely ground sample such as flue-dust and chamber muds is perfectly decomposed by treating with an acid mixture, consisting of one part of water, two parts of HCl and HNO_3, respectively, and four parts of sulfuric acid, being mixed in the named order. Complete evaporation is important for complete isolation of lead, silica, bismuth and others. Then the cooled residue is disintegrated with 1N hot H_2SO_4, to which a few drops of H_2O_2 is added to reduce any $Tl^{\cdots}$ that may have been formed. After a while it is filtered to separate the insoluble matter. Complete extracting with the sulfuric acid is necessary for success. To neutralize the greater part of the free acid, the extracted solution is treated with concentrated sodium hydroxide solution. At this stage a precipitate is formed, which diss-

Table 1. Acid Concentration

Acid Concentration	$Co(NH_3)_6TlCl_6$ (g.)	Tl-found (g.)	Tl-present (g.)	Difference (g.)	Remarks
0.33N HCl	0.0284	0.01004	0.01002	+0.00002	added some NH_4Cl
0.66N HCl	0.0288	0.01018	0.01002	+0.00016	instantly pptd.
1.32N HCl	0.0283	0.01000	0.01002	−0.00002	,,
1.98N HCl	0.0238	0.01018	0.01002	+0.00016	
3.30N HCl	0.0298	0.01060	0.01002	+0.00058	luteo salt pptd.
0.82N HNO_3	0.0265	0.00937	0.00970	−0.00033	
2.26N HNO_3	0.0230	0.01025	0.00970	+0.00055	
3.86N HNO_3	0.0064	0.00227	0.00970	−0.00743	precipitate dissolved
5.46N HNO_3	0.0005	0.00047	0.00970	−0.00923	,,
1.02N H_2SO_4	—	—	0.00970	−0.00970	not precipitated
2.46N H_2SO_4	0.0237	0.00837	0.00970	−0.00133	
4.26N H_2SO_4	0.0168	0.00594	0.00970	−0.00376	
7.86N H_2SO_4	—	—	0.00970	−0.00970	,,

cipitation as sometimes happen especially with sulfuric acid or nitric acid solution owing to the amount of chloride ion present being insufficient to form $TlCl_6'''$. But this difficulty is easily removed by the addition of ammonium chloride as shown in Table 2 and the range of acid concentration is safely extended to about 3N. Moreover the determination can be made in any kind of acid medium.

It was also found that the addition of about 0.5 g. ammonium chloride is responsible for the perfect change of thallium ion into $TlCl_6'''$. Furthermore it makes possible the determination of thallium as slight as 5 mg. gravimetrically indeterminable by any other method up to date. Thallium is not easily oxidized with nitric acid alone, but its complete oxidation can be effected in the presence of any chloride ion. As there is a tendency that hydrolysis of thallic nitrate or sulfate will occur, especially when hot, the formation of $TlCl_6'''$ is sometimes difficult. But ammonium chloride removes also these difficulties. Consequently the addition of ammonium chloride newly recommended is remarkably useful in this instance, especially with sulfuric acid solution most commonly used.

Table 2. Effectiveness of the Addition of NH_4Cl

Acid Concentration	HCl (ml.)	NH_4Cl (g.)	Tl-complex (g.)	Tl-found (g.)	Tl-present (g.)	Difference (g.)
2.0N H_2SO_4	15	0.5	0.0324	0.01145	0.01149	−0.00004
2.0N H_2SO_4	15	none	0.0311	0.01099	0.01149	−0.00050
2.0N H_2SO_4	none	0.5	0.0325	0.01149	0.01149	−0.00000
2.0N H_2SO_4	none	none	0.0229	0.00081	0.01149	−0.01068
1.9N HNO_3	15	0.5	0.0326	0.01152	0.01149	+0.00003
1.9N HNO_3	15	none	0.0235	0.01007	0.01149	−0.00142
1.9N HNO_3	none	0.5	0.0308	0.01089	0.01149	−0.00060
1.9N HNO_3	none	none	0.0237	0.00105	0.01149	−0.01044
2.5N H_3PO_4	none	0.5	0.0301	0.01064	0.01032	+0.00032
2.5N H_2SO_4	5	0.5	0.0304	0.01075	0.01032	+0.00043
2.5N H_2SO_4	5	none	0.0232	0.00997	0.01032	−0.00035

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solves slightly on stirring. The remainder of the free acid is accurately neutralized with sodium carbonate solution. The end point is easily perceived by the slow evolving of carbon dioxide gas.

The solution is then heated to boiling and sulfur dioxide is passed until it cools to room temperature. The precipitate, if present, is filtered off. In this condition, thallium exists as thallous ion. Through the procedure until this stage, lead, bismuth, tin, antimony, and mercury etc. have already been removed. Then the solution is acidified to 1N acid concentration with hydrochloric acid and heated to expel any traces of sulfur dioxide. The solution is now ready for oxidation and precipitation in the same way as has been given in the general procedure, including the washing and weighing.

(*b*) *Interference.* Interferences by several common ions were tested with proposed method, but no serious interference has been noted under the conditions recommended above. The results have a tendency to be slightly higher than the true value as shown in Table 4. The chief sources of error arise from incomplete neutralization and incomplete treatment with sulfur dioxide or from insufficient washing neglecting to remove any contaminable matter. For example, the luteo salt reacts with cadmium to form an orange-colored complex compound. For best results, twice the amount of washing solution already mentioned as required for washing is necessary. However no fatal dissolution of the thallium complex compound was perceptible due to profuse washing. It is evident from table 4 that minute amounts of thallium as small as 10 mg. or even less can be determined in the presence of many other ions a thousand times greater in quantity.

Discussion. The iodide or chromate method was widely used among the many methods. However, the former tends to give slightly lower results than the real value and requires about 18 hours for the complete precipitation owing to its colloid forming tendency. The latter has an advantage over the former in some respects, but it requires also at least 12 hours for standing and is fatally defective in that thallium chromate formed is by nature perceptibly soluble. Moreover the factor is larger than double that of the proposed method. Thus it is easily conceivable that the determination of such minute amounts of thallium less than 50mg. per 100 ml.[9] is impossible. But in this respect, the proposed method was found to be definitely satisfactory as shown in Table 4, even in the case of a minute amount as 5 mg. per 100 ml.

Mach *et al*[10] had found that the determination of thallium in small

(9) J. Bondanar and A. Tereniyi, *Z. anal. Chem.*, **69** (1926), 32.

(10) F. Mach and W. Lepper, *Z. anal. Chem.*, **69** (1926), 42; R.J. Meyer, *Z. anorg. allgem. Chem.*, **24** (1900). 364.

amounts impossible with the above method, can be undertaken as Tl_2O_3. But even the use of this method in flue-dust is definitely unsatisfactory due to the analogous reasons as mentioned above with the chromate method.

Five most widely used methods involving these methods were discussed by Strecker *et al*[11] who concluded that Tl(I)-Co(III)-nitrite is most satisfactory. But the minimum amount determined was 0.1 g. Tl and

Table 4. Determination in the Presence of Other Metals

Ion	Salt used (Amount in g.)	$Co(NH_3)_6(TlCl_6)$ (g.)	Tl-found (g.)	Tl-present (g.)	Diff. (g.)	Precipitate
K^+	KCl (10)	0.0326	0.01152	0.01149	+0.00003	
Na^+	NaCl (10)	0.0324	0.01145	0.01149	−0.00004	
K^+Al^{+++}	Alum (10)	0.0282	0.00997	0.01000	−0.00003	
SiO_3''	Na_2SiO_3 (5)	0.0300	0.01060	0.01032	+0.00028	filtered
		0.0290	0.01025	0.01032	−0.00007	,,
Ca^{++}	$CaCl_2$ (5)	0.0287	0.01014	0.01003	+0.00011	
Ti^{++++}	$Ti(SO_4)_2$	0.0284	0.01004	0.01003	+0.00001	,,
Cr^{+++}	$CrCl_3$ (5)	0.0284	0.01004	0.01003	+0.00001	
Mn^{++}	$MnSO_4$ (5)	0.0301	0.01064	0.01032	+0.00032	
		0.0291	0.01029	0.01032	−0.00003	
Fe^{+++}	$FeCl_3$ (10)	0.0304	0.01071	0.01032	+0.00039	
		0.0326	0.01152	0.01149	+0.00003	
Co^{++}	$CoSO_4$ (5)	0.0296	0.01046	0.01032	+0.00014	
Co^{++}	$CoCl_2$ (5)	0.0286	0.01011	0.01003	+0.00008	
Ni^{++}	$NiCl_2 6H_2O$ (5)	0.0289	0.01022	0.01003	+0.00019	
		0.0299	0.01053	0.01032	+0.00020	
Cu^{++}	$CuSO_4 5H_2O$ (10)	0.0283	0.01000	0.01000	0.00000	
Zn^{++}	$Zn(C_2H_3O_2)$ (5)	0.0284	0.01034	0.01032	+0.00002	
		0.0330	0.01166	0.01149	+0.00017	
AsO_3'''	As_2O_3 (5)	0.0287	0.01014	0.01003	+0.00011	
MoO_4''	$(NH_4)_6Mo_7O_{24}4H_2O$ (5)	0.0284	0.01004	0.01003	+0.00001	,,
Ag^+	$AgNO_3$ (10)	0.0281	0.00993	0.01000	−0.00007	,,
Co^{++}	$Cd(NO_3)_2$ (10)	0.0284	0.01004	0.01003	+0.00001	
Sn^{++}	$SnCl_2 2H_2O$ (5)	0.0283	0.01000	0.01000	0.00000	,,
		0.0261	0.00923	0.00986	−0.00063	,,
Sb^{+++}	$SbCl_3$ (5)	0.0279	0.00986	0.01000	−0.00014	,,
Ba^{++}	$BaCl_2 2H_2O$ (10)	0.0259	0.00916	0.00986	−0.00070	,,
		0.0290	0.01024	0.01000	+0.00024	,,
Hg^+	$Hg_2(NO_3)_2$ (5)	0.0289	0.01022	0.01000	+0.00022	,,
Pb^{++}	$Pb(NO_3)_2$ (10)	0.0284	0.01004	0.01000	+0.00004	,,
Bi^{+++}	$BiCl_3$ (5)	0.0283	0.01000	0.01000	0.00000	,,
Mg^{++}	$Mg(NO_3)_2 6H_2O$ (5)	0.0283	0.01000	0.01000	0.00000	
Zn^{++}	$ZnSO_4$ (5)	0.0329	0.01163	0.01149	+0.00014	
Zn^{++}	$Zn(C_2H_3O_2)_2$ (5)	0.0320	0.01141	0.01132	+0.00009	
NO_3'	HNO_3	0.0326	0.01152	0.01149	+0.00003	
SO_4''	H_2SO_4	0.0324	0.01145	0.01149	−0.00004	
PO_4'''	H_3PO_4	0.0286	0.01011	0.01000	+0.00011	
PO_4'''	$(NH_4)_2HPO_4$	0.0301	0.01064	0.01032	+0.00032	

* Precipitate formed when treated with sulfur dioxide.

(11) W. Strecker and P. de la Pena, *Z. anal. Chem.*, **67** (1926), 257.

the factor exceedingly large (0.6469). Owing to this reason and for the necessity of preliminary separation, it is also unsatisfactory for application to flue-dust. The thionalide method, recommended in the determination of such a little amount as 5 mg., cannot be used in the presence of many metals frequently occurring in flue-dust. Matsubara and Kuwabara[12] found that the thionalide method and chromate method can only give an approximate quantitive result in such a sample because of the presence of many metals. From such standpoints it may be concluded that the proposed method is remarkably superior to the above mentioned and can be easily applied for the determination of minute amounts of thallium.

The superiority will be summarized in the following:

(a) The addition of ammonium chloride realizes the determination of 5 mg. thallium in any mineral acid medium with fairly good results.

(b) The proposed method is time-sparing, usually only requiring two or three hours from the time the sample is brought into solution. It simplified the procedure for the determination particularly in such a substance as flue-dust which is usually in association with numerous other metals. The veracity of this concluded superiority can be proved by comparing it with the thionalide method having the least factor among many of the other methods already mentioned.

(c) It does not require a troublesome separation, thus eliminating another possible source of error which results from the minute amount of thallium being absorbed on voluminous and absorptive precipitates of iron, manganese etc. as is frequently the case noted in other methods.

(d) The factor is the smallest among any other gravimetric method, thus making possible the determination of a minute amount of thallium present in the sample such as flue-dust which is impossible with any other method.

The author wishes to express his sincere gratitude to Prof. Kenjiro Kimura for advising him to undertake this study and for many valuable suggestions. The author also expresses his thanks to Miss F. Ueda who has taken great interest in this experiment and has rendered invaluable help.

Chemical Institute, Faculty of Science,
Tokyo University.

(12) S. Matsubara and T. Kuwabara, *J. Min. Soc. Japan*, **61** (1945), 7.

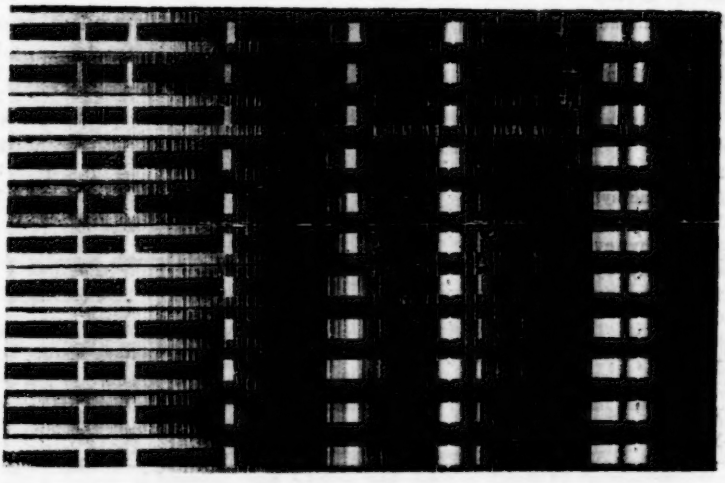
Analysis of Straight-Run Gasolines by Means of the Raman Effect.

By San-ichiro MIZUSHIMA, Shizuo NAKAMURA, and Tsuneo TOBIYAMA.

(Received May 24, 1949.)

The analysis of hydrocarbon mixtures such as gasolines is by no means easy, so far as we adopt only the ordinary chemical procedure. This difficulty can, however, be overcome by the application of Raman effect, because each hydrocarbon molecule has its characteristic Raman lines whose frequencies are not appreciably modified by the presence of other hydrocarbon molecules. The present note deals with the analysis of some straight-run gasolines (end point 150°C) of Sumatra and Borneo by this optical method.

Each of the five samples of straight-run gasolines (Sanga-Sanga, Rantau, Pentopo, Seria, and Miri) were distilled carefully through a long column into 20 ~ 25 fractions and for each fraction the Raman spectrum was measured (see the photograph). Two spectrographs of our own construction were used in this measurement, the larger one having three prisms of the height of 10 ~ 15 cm. From the determination of the in-



The Raman Spectra of Rantau-gasoline (for the fractions of ~30°C, 30~55°C, 55~60°C, 60~65°C, 65~70°C, 70~75°C, 75~80°C, 80~85°, 85~90°C, 90~95°C and 95~100°C)

tensity of Raman lines assigned to a definite hydrocarbon[1] these samples were analysed as shown in the table. The components thus determined

Table

Component \ Gasoline	Sanga Sanga	Rantau	Pentopo	Seria*	Miri*
Isobutane		T	T	0.1	0.1
n-butane		T	1	0.1	0.1
2, 2-Dimethyl propane		T	T	T	T
Isopentane	2	1	1	0.5	0.5
n-Pentane	2	1	1	1	1
2, 2-Dimethyl butane	1	1	T	0.2	0.5
Cyclopentane	1	1	1	0.5	1
2, 3-Dimethyl butane	1	2	1	0.3	0.5
2-Methyl pentane 3-Methyl pentane	4	2	4	2.5	2.5
n-Hexane	3	2	5	2.5	1.5
Methyl cyclopentane	2	2	3	1	1.5
2, 2-Dimethyl pentane	1	1	T	0.5	0.1
Benzene	7	1	2	1.8	1.5
Cyclohexane	6	5	3	5	8
2, 4-Dimethyl pentane	2	1	1	0.4	0.1
2, 2, 3-Trimethyl butane		T	T	T	0.05
3, 3-Dimethyl pentane	1	1	T	0.3	0.1
2, 3-Dimethyl pentane		1	1	0.2	0.1
2-Methyl hexane	1	3	3	1.5	1
3-Metoyl hexane			3	1	1
3-Ethyl pentane			T	0.1	0.1
n-Heptane	2		9	4	5
2, 2, 4-Trimethyl pentane				T	T
Methyl cyclohexane	11	10	7	14	17
1, 2-Dimethyl cyclopentane	2	2	1	0.1	1.5
Toluene	14	6	7	6	5.5
2, 2, 3-Trimethyl pentane				T	T
2, 3, 4-Trimethyl pentane				T	T
1, 1-Dimethyl cyclohexane				0.5	1
1, 3-Dimethyl cyclohexane	4	4	2	3.5	7
1, 4-Dimethyl cyclohexane	2	4	2	2.5	5
1, 2-Dimethyl cyclohexane		3	2	1.5	5
n-Octane	3		3	3	2
Ethyl benzene	1	1	1	2	1
p-Xylene	2	2	2	2	2
m-Xylene	6	7	5	6	5
o-Xylene	1	4	2	1.5	2
n-Nonane				2	0.5

† T means "trace"

* Seria and Miri gasolines were analysed more carefully than the other three.

(1) As to new data of the Raman spectra of pure *n*-hydrocarbons see Mizushima and Simanouti: J. Am. Chem. Soc. **71** (1949), 1320.

amount to 70 ~ 80 per cent of the original gasolines. The remaining 30 ~ 20 per cent corresponds to the hydrocarbons whose Raman spectra have not been reported.

We thank Miss H. Shirakawa and Mr. M. Morikawa for their assistance in this measurement.

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On the Molecular Association in Amides as Revealed by Their Near-Infrared Absorption Spectra.

By Masamichi TSUBOI.

(Received May 25, 1949.)

As a part of his investigations on the hydrogen bondings in compounds with the peptide linkage, the present writer made some observations of near-infrared absorption spectra of *N*-methylacetamide and δ-valerolactam, two representatives of compounds with the said linkage, one being of the single chain type and the other of the cyclic type. Some of the results obtained are of interest in relation to the general problems of association in amides. It is the purpose of this paper to give the results of the writer's observations of the above-mentioned two substances, and to present a view as to their molecular configurations, and then to discuss some problems of association in amides.

Experimental.

The infrared absorption in the 2.6~3.6 μ region were measured of *N*-methylacetamide ($CH_3CONHCH_3$) and δ-valerolactam ($\begin{pmatrix} CH_2\text{-}CH_2\text{-}CH_2 \\ | \quad\quad | \quad\quad | \\ CH_2\text{-}CO\text{-}NH \end{pmatrix}$) in liquid state and in carbon tetrachloride solutions.

The optical system of the spectrometer used in the investigation consists of concave mirrors and 60° quartz prism. This was used in conjunction with the thermocouple and galvanometer system. As the source of radiation a tungsten filament lamp in a quartz bulb, running at 80 volts and 1.6 amperes, was used. To determine the wave-lengths of the absorption bands the following standards were used: 2.769 μ band of phenol, 3.233 μ and 3.295 μ bands of benzene, and 3.416 μ and 3.508 μ bands of cyclohexane, in dilute carbon tetrachloride solutions.

The samples of *N*-methylacetamide and δ-valerolactam for this investigation were prepared respectively by K. Mayumi and I. Okano and by K. Hoshino and S. Yumoto, to whom the writer's thanks are due. These were subjected several times to vacuum distil-

lation. The melting points determined with the distilled samples were: 28°C for *N*-methylacetamide and 40°C for δ-valerolactam. Carbon tetrachloride used as the solvent, which was washed with water, dried over phosphorus pentoxide, and distilled, was confirmed to give no absorption band due to impurities in the said region of the spectra.

Results and Their Interpretations.

In Tables 1 and 2 certain results obtained by the present measurements are shown. (Cf. Figs. 1 and 2.)

Table 1. Infrared absorptions in 3 μ region of *N*-methylacetamide.

States		Temperatures (°C)	Wave-lengths (in μ) of absorption maxima, with molar absorption coefficients in parentheses	
			Free *N-H* band	*N-H* association bands
In CCl_4 solution				
Concentrations	0.0014 mol/l.	15	2.88 (17)	
	0.0032	15	2.88 (14)	2.97 (9)
	0.031	13	2.88 (12)	3.09 (33) 3.22 (18)
	,,	40	2.88 (14)	2.97 (15)
	0.079	16	2.88 (9)	3.03 (35) 3.22 (27)
	,,	39	3.88 (9)	2.99 (25) 3.22 (12)
Liquid state		30		3.03 (73) 3.22 (54)

Table 2. Infrared absorptions in 3 μ region of δ-valerolactam.

States		Temperatures (°C)	Wave-lengths (in μ) of absorption maxima, with molar absorption coefficients in parentheses	
			Free *N-H* band	*N-H* association bands
In CCl_4 solution				
Concentrations	0.0015 mol/l.	15	2.92 (11)	3.11 (41) 3.24 (26)
	,,	50	2.92 (16)	3.11 (18) 3.24 (10)
	0.016	13	2.92 (6)	3.11 (46) 3.24 (38)
	,,	50	2.92 (12)	3.11 (18) 3.24 (12)
	0.023	13	2.92 (4)	3.11 (49) 3.24 (40)
	,,	40	2.92 (7)	3.11 (28) 3.24 (23)
Liquid state		40		3.09 (55) 3.23 (30)

As is seen from the tables, the absorption intensity for the band at 2.88 μ of *N*-methylacetamide in carbon tetrachloride solution increases with lowering of the concentration of the solution and with rise of its temperatures; while the reverse was observed as to the absorption intensities for the bands at $2.97 \sim 3.03\,\mu$ and $3.22\,\mu$. This fact may be explained, as usual, by assigning the band at $2.88\,\mu$ to the N-H vibration of the free molecules and the bands at $2.97 \sim 3.03\,\mu$ and $3.22\,\mu$ to the N-H vibrations of the associated molecules. In the same way, the absorption at $2.92\,\mu$ of δ-valerolactam may be assigned to the N-H vibration in the free molecules of δ-valerolactam, and the bands at $3.11\,\mu$ and $3.24\,\mu$ to the N-H vibrations in its associated molecules. It is thus shown

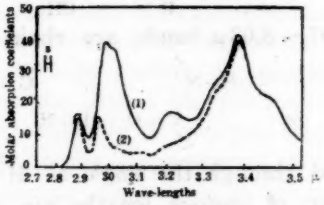


Fig. 1. Absorption curves of *N*-methylacetamide in CCl_4 solution (0.031 mol/l), (1) at 13°C, (2) at 40°C. (s: Slit width)

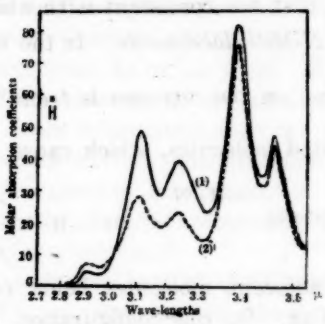


Fig. 2. Absorption curves of δ-valerolactam in CCl_4 solution (0.023 mol/l), (1) at 13°C, (2) at 40°C (s: Slit width)

that the molecules of *N*-methylacetamide as well as those of δ-valerolactam associate through their intermolecular N-H····O bondings. There are, however, remarkable differences, as enumerated below, between these two kinds of molecules in the absorption intensities and the positions of the bands in their 3 μ region absorption spectra, indicating the difference in their modes of molecular association.

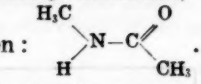
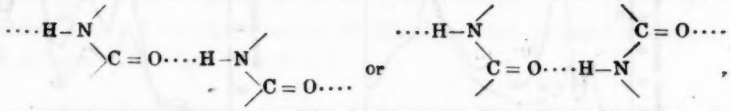
(i) The N-H band (at 2.92 μ) of the free molecule of δ-valerolactam is located at a longer wave-length than that (at 2.88 μ) of the free molecule of *N*-methylacetamide. This shows that the N-H bonding in the δ-valerolactam molecule is perturbed a little to weaken the bonding, in comparison with that in the *N*-methylacetamide molecule, even in the monomer state—i. e. intramolecular perturbation.

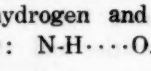
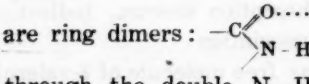
(ii) The tendency of molecular association in carbon tetrachloride solutions is stronger for δ-valerolactam than for *N*-methylacetamide. Thus, even at such a low concentration as 0.0015 mol/l., δ-valerolactam molecules are for a major part in associated state, while at about the same concentration (0.0014 mol/l.), *N*-methylacetamide molecules are in isolated state. In other words, the change in free energy (ΔF) accompanying the dissociation of dimer is much greater for δ-valerolactam than for *N*-methylacetamide.

(iii) With lowering of concentration of the solution or with rise of its temperature, there takes place the shift of the absorption maxima of the N-H association band of *N*-methylacetamide, from 3.03 μ to 2.97 μ, but no such shift of the absorption maxima of the bands of δ-valerolactam. This fact indicates that there can be only one kind of associated molecules of δ-valerolactam, while there are more than one kind of associated molecules of *N*-methylacetamide, in the respective solutions.

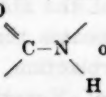
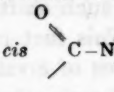
The molecular configurations of *N*-methylacetamide and δ-valerolac-

tam that are consistent with what have been stated above are as follows:

N-Methylacetamide. In the molecule of *N*-methylacetamide, the hydrogen on the nitrogen is *trans*[1] to the oxygen:  . The associated molecules, which cause the 2.97 ~ 3.03 μ bands, are chain type polymers:  , the adjacent molecules being combined through the single N—H····O bonding. On this configuration, polymers of various lengths are possible, in consistence with the indication that there are more than one kind of associated molecules of *N*-methylacetamide.

δ-Valerolactam. In the molecule of δ-valerolactam, the hydrogen on the nitrogen is *cis* to the oxygen:  . The intramolecular perturbation, mentioned in (i), may be attributed to the attraction between these hydrogen and oxygen atoms, viz. the intramolecular hydrogen bonding: N-H····O. The associated molecules, which cause the 3.11 μ band, are ring dimers:  , the two molecules being combined through the double N—H····O bondings. This configuration is consistent with the indication that there is only one kind of associated molecules of δ-valerolactam. Further, intermolecular double bondings in δ-valerolactam, in contrast to the single bonding in *N*-methylacetamide, account for the greater dissociation energy in the latter.

A few words may be added here concerning the 3.22 μ association band of *N*-methylacetamide. In its pure liquid state, this band is comparable in absorption intensity with the 3.03 μ band. It is not apparent to the writer, to what this 3.22 μ band is to be assigned. But, since the Raman spectra[2] in the region of the skeletal frequencies of the *N*-methylacetamide molecule show that in its pure liquid state only one skeletal structure is possible, and moreover since the writer's observation reveals that the molecule of *N*-methylacetamide, is *trans*, this 3.22μ band must be due to something in the associated molecules consisting

(1) In a molecule with the —CONH— linkage, the C—N bond has some double-bond character, due to resonance, O=C—NH— ↔ $\bar{O}$—C=$\overset{+}{N}$H—; and this tends to cause the molecule to take the coplaner configuration, i.e. *trans*  or *cis*  configuration.

(2) S. Mizushima, T. Simanouti, S. Nagakura, K. Kuratani, M. Tsuboi, H. Baba and O. Fujioka. (To be published.)

only of *trans* molecules. Buswell and others[3] assigned the 3.22 μ band of monosubstituted amides to the ring dimer structure of their associated molecules, componental monomer of which must be of *cis* form. In the light of what has been just mentioned, this view is not appropriate for the 3.22 μ band of *N*-methylacetamide. More information than we have at present is needed before we can discuss further this question. A fact, perhaps worthy of mention in the present connection, is that neither the Raman line for this band nor the infrared absorption band corresponding to its overtone has ever been observed.[2]

As to the assignment of the 3.24 μ association band of δ-valerolactam too, nothing can be said at present. This, as well as the above, is a question yet to be solved.

Discussion on Some General Problems of Molecular Association in Amides.

Some general problems of association in amides will now be discussed. In this field the studies made by A. M. Buswell and his collaborators[3][4] are important. Some of their data, along with the present writer's, are given in Table 3. It is chiefly on these data that the following discussion is based.

Table 3.

3μ region infrared absorptions of amides in CCl_4 solution.

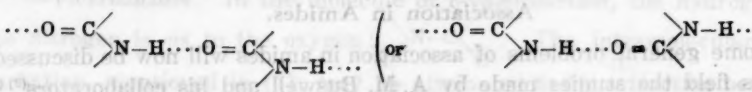
	Substances	Wave-lengths of free *N-H* bands (in μ)		Wave-lengths of *N-H* association bands (in μ)		Molar absorption coefficients of *N-H* association bands	Observers
Monosubstituted Amides	*N*-Methylacetamide	2.88		2.97→3.03 (b)	3.22	0 9 (2.97 μ)	at 0.0014 mol/l. at 0.0032 mol/l. M. Tsuboi
	N-Ethylacetamide	2.89		2.97→3.03 (a) (b)	3.22	6 (a) (2.97 μ)	at 0.002 mol/l. A.M. Buswell and others[4]
	N-Cyclohexylacetamide	2.90		2.97→3.04 (b)	3.23	7 (2.97 μ)	at 0.002 mol/l. A.M. Buswell and others[4]
Disubstituted Amides	Acetamide	2.83	2.925	3.03	3.145	17 (3.145 μ)	at 0.002 mol/l. A.M. Buswell and others[3][4]
	Propionamide	2.83	2.925	3.03	3.15	8(a)(3.03 μ) 14(a)(3.15 μ)	at 0.002 mol/l. A.M. Buswell and others[4]
	n-Valeramide	2.83	2.92	3.03	3.14	19 (3.14 μ)	at 0.002 mol/l. A.M. Buswell and others[3][4]
	Benzamide	2.83	2.925	3.03	3.15	11(a)(3.03 μ) 25(a)(3.15 μ)	at 0.002 mol/l. A.M. Buswell and others[4]
	δ-Valerolactam	2.92		3.11	3.24	41 (3.11μ)	at 0.0015 mol/l. M. Tsuboi

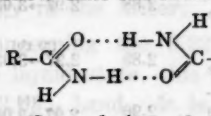
(a) Read from Buswell's curves.
(b) The arrow indicates the shifting of the band on raising the concentration of the solution.

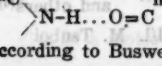
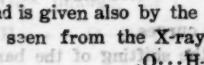
(3) A.M. Buswell, J.R. Dowing and W.H. Rodebush, *J. Am. Chem. Soc.*, **62** (1940), 2759.

(4) A. M. Buswell, W. H. Rodebush, and M.F. Roy, *J. Am. Chem., Soc.*, **60** (1938), 2444.

Structures of Associated Molecules of Amides. *Monosubstituted Amides.* The inspection of Table 3 reveals that *N*-methylacetamide, *N*-ethylacetamide, and *N*-cyclohexylacetamide are closely similar to each other in their 3 μ region absorptions in carbon tetrachloride solution. Thus, they are nearly equal with respect to the wave-lengths of N—H absorption bands of free and associated molecules, with respect to the absorption intensities of association bands, and with respect to the shifting of the 2.97 ~ 3.04 μ band with concentrations of the solutions. These show that *N*-methylacetamide, *N*-ethylacetamide, and *N*-cyclohexylacetamide are alike in the molecular configurations, both in the single and associated states. In general it may be said that: the molecules of the ordinary monosubstituted amides RCONHR′ are of the *trans* form (probably due to the steric repulsion between R and R′ in each molecule), and on association they form chain polymers:



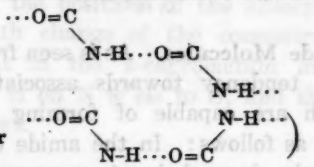
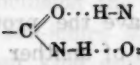
Unsubstituted Amides. Unsubstituted amides show N—H association bands at 3.03 μ and 3.14 ~ 3.15 μ. Of these two bands the former is located near the 2.97 ~ 3.03 μ band of *N*-methylacetamide and the latter near the 3.11 μ band of δ-valerolactam. Moreover, at relatively low concentration (e. g. 0.002 mol/l.) in their carbon tetrachloride solution, the absorption intensity of the 3.14 ~ 3.15 μ band of unsubstituted amides is stronger than that of the 3.03 μ band; likewise the absorption intensity of the 3.11 μ band of δ-valerolactam is stronger than that of the 2.97 μ band of *N*-methylacetamide. These facts show that the molecules $RCONH_2$ associate at first to form ring dimers:  through double N—H····O bondings (the hydrogen here being that one of the two, which is *cis* to the oxygen in each molecule), and then polymers through single N—H····O bonding (the hydrogen here being the other one of the two).

N. B. The absorption band at 3.11~3.15 μ might be due to the configuration  in the ring dimer structure rather than the ring dimer structure itself, for, according to Buswell[3], the 3.14 μ band is given also by the crystal of acetamide, in which the constituent molecules, as may be seen from the X-ray analysis by F. Senti and D. Harker[5], are bonded so that the configuration  is formed but not the ring dimer structure.

(5) F. Senti and D. Harker, *J. Am. Chem. Soc.*, **62** (1940), 2008.

N-H Stretching Vibrations in the Associated Molecules of Amides. The above conclusion concerning the relation between the frequencies of N—H stretching vibrations in amides and the configurations of their associated molecules may be summed up in Table 4. It is to be noted that the difference in frequencies of N—H stretching vibrations (in corresponding linkages) between $C-CO-NH_2$ and C—CO—NH—C is comparatively small.

Table 4.

Frequencies of N-H stretching vibrations	$C-CO-NH_2$	C-CO-NH-C
(A) in single N—H···O=C	——	3360 cm.$^{-1}$ (2.97 μ)
(B) in 	3300 cm.$^{-1}$ (3.03 μ)	3360~3280 cm.$^{-1}$ (2.97~3.04 μ)
(C) in 	3180~3170 cm.$^{-1}$ (3.14~3.15 μ)	3210 cm.$^{-1}$ (3.11 μ)

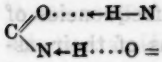
The fact that the frequencies are lower in (B) than in (A), as shown in Table 4, may be explained as follows: In the molecule of an amide, there is an electric polarity due to resonance, $O=C-NH- \leftrightarrow \bar{O}-C=\dot{N}H-$. This polarity must increase, if the oxygen of this molecule accepts the hydrogen (or proton) of another molecule, and accordingly the electronegativity of the nitrogen must increase too. Hence, the N—H···O bonding must be stronger in the associated molecules of the polymer (B) than in those of the non-ring type dimer (A), and the N—H bond in the former must be weaker than that in the latter. This explains why the frequencies of N—H stretching vibrations is lower in the former than in the latter.

That the frequencies are lower in (C) than in (B) and (A) (Table 4) may be explained as follows: Let the potential energy V of the system (C) be

$$V = \frac{1}{2} k_1(q_1^2 + q_2^2) + k_2 q_1 q_2,$$

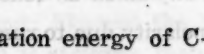
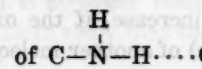
where q_1 and q_2 are the displacements (at a given time) from equilibrium in the stretching vibrations of the two N—H's in the system, the signs of q_1 and q_2 being taken as positive for stretching and as negative for contraction; k_1 the force constant of each N—H; and k_2 the force constant corresponding to the interaction between the two N—H's. Then

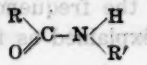
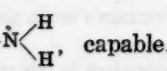
we obtain the frequency ν, for the infrared-active vibration:



$$\nu = \frac{1}{2\pi c}\sqrt{\frac{k_1 - k_2}{m}},$$

where m is the reduced mass of N—H. In (C), the Coulomb repulsion between the two hydrogens (or protons) is probably the most effective interaction between the two N—H's. If so, k_2 must be positive, because the stretching of the distance of one N—H in the system (C) tends to contract the distance of the other N—H, while k_2 must be nearly zero in (B) and (A). This explains why the frequencies are lower in (C) than in (B) and (A).

Association Tendencies of Amide Molecules. As is seen from Table 3, δ-valerolactam shows a stronger tendency towards association than unsubstituted amides, both of which are capable of forming the ring dimer. This fact may be explained as follows: In the amide or lactam molecule, the nitrogen atom, positively charged due to resonance, would attract the electrons of the hydrogen atoms and leave the protons bare to be attached to negatively charged oxygen atoms of another molecule. This process would be more effective (per one N—H) in the case where only one hydrogen is on the nitrogen than in the case where two hydrogens are on it. Hence, the dissociation energy of  bonding must be greater than that of  bonding. This explains the difference in association tendencies of the two kinds of molecules.

Buswell and his collaborators[4] indicated that the monosubstituted amides in general show a smaller tendency towards association than the unsubstituted amides. This difference in associasion tendencies is, according to the present writer's view, due to the difference in molecular structures of these two kinds of amides, the one,  being incapable of forming ring dimers, the other,  capable. It is rather probable, however, that the dissociation energy per one N—H···O bonding is greater in monosubstituted amides (and lactams) than in unsubstituted amides.

Supplement.

While this paper was in press, the writer made some observations on the infrared absorptions of ε-caprolactam ($\begin{pmatrix} CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2 \\ CO\text{-}NH\text{-}CH_2 \end{pmatrix}$), another example of the cyclic compounds with the peptide linkage, and found the following facts: (1) ε-Caprolactam shows in its carbon tetrachloride solution the free N–H absorption band at 2.90μ and the N–H association bands at 3.09μ and 3.23μ. (2) ε-Caprolactam has a stronger tendency of molecular association than monosubstituted amides, the molar absorption coefficient at 3.09μ of ε-caprolactam being as great as 22 even in such a dilute carbon tetrachloride solution as 0.0007 mol/l. (3) No shifting occurs in the positions of the absorption maxima of the N–H association bands with change of the concentration of the solution. These facts show that in the ε-caprolactam molecule, as in the δ-valerolactam molecule, H on N is *cis* to O, and that the molecules associate to form ring dimers.

In conclusion the writer wishes to express his sincere thanks to Professor San-ichiro Mizushima and Assistant Professor Takehiko Simanouti for their kind guidance throughout the present work.

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Tokyo University.